

## Modeling of Sago Starch Hydrolysis Using Glucoamylase (Pemodelan Hidrolisis Kanji Sagu dengan Glukoamilase)

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### ABSTRACT

*A mathematical model based on Michaelis-Menten kinetics was proposed and used to simulate the sago starch hydrolysis and the remaining sago starch concentration. The simulation model was tested with comparison to the real experimental results. The glucoamylase enzyme (E.C. 3.2.1.3) from *Aspergillus niger* was employed in the present study to hydrolyze the substrate i.e. sago starch into reducing sugars. The experiment was performed in a 2 L stirred tank reactor. The quantitative effects such as temperature, substrate concentration, enzyme amount, pH and agitation speed were set to optimal value at 61 °C, 1.0 gL<sup>-1</sup>, 0.2 Uml<sup>-1</sup>, pH4.5 and 100 rpm, respectively. The computer software, Polymath® 6.0 was employed in this research. The numerical method based on Runge-Kutta Fehlberg 45 was used to solve the system of non-linear ordinary differential equations (ODEs) of sago starch hydrolysis. The model prediction was well agreed with experimental results where the high values of regression coefficient, R<sup>2</sup> = 0.9643 was obtained.*

*Keywords: Glucoamylase; Michaelis-Menten kinetics; sago starch; reducing sugar; simulation model*

### ABSTRAK

*Model matematik berasaskan kinetik Michaelis-Menten telah dicadangkan dan digunakan untuk mensimulasikan hidrolisis kanji sagu dan kepekatan kanji sagu yang tertinggal. Model simulasi ini telah diuji dengan perbandingan keputusan eksperimen. Enzim glukoamilase (EC 3.2.1.3) daripada *Aspergillus niger* telah pun diguna pakai dalam kajian ini bagi tujuan menghidrolisis substrat iaitu kanji sagu ke gula penurunan. Eksperimen ini dijalankan dalam tangki reaktor 2 L. Kesan kuantitatif seperti suhu, kepekatan substrat, jumlah enzim, pH dan kelajuan perolakan telah dilaraskan pada nilai optimum iaitu masing-masing 61 °C, 1.0 gL<sup>-1</sup>, 0.2 Uml<sup>-1</sup>, pH4.5 dan 100 rpm. Perisian komputer Polymath® 6.0 telah digunakan dalam penyelidikan ini. Kaedah berangka berdasarkan Runge-Kutta Fehlberg 45 juga digunakan bagi menyelesaikan sistem persamaan pembezaan biasa tidak linear untuk hidrolisis kanji sagu. Jangkaan keputusan model ini adalah bertepatan dengan keputusan eksperimen dengan nilai pekali regresi yang tinggi, R<sup>2</sup> = 0.9643 telah diperolehi.*

*Kata kunci: Glukoamilase; kinetik Michaelis-Menten; kanji sagu; gula penurunan; model simulasi*

### INTRODUCTION

At the end of the 20th century, in Asia Pacific and South East Asia region, the starch extracted by sago palm is estimated about 60 million tons per year (Wang et al. 1996). Malaysia, one of the sago starch producing country contributed about 25000-40000 tons of sago products annually (Singhal et al. 2008). Sago starch which is widely used in food and non-food application brings great economic impact to Malaysia. There is about RM40.4 million yearly incomes based on food-grade sago starch alone (Wee et al. 2011a). Due to the abundance of sago palm and its natural renewable properties, the sago starch powder may be served as fermentable sugars with accomplished by using saccharifying enzymes on large scale. The previous practice of sago starch hydrolysis process is involved using acid and now has been replaced by the enzymatic process (Adinarayan & Suren 2005). Glucoamylase enzyme derived from *Aspergillus niger* is one of the saccharifying enzyme and has been used to hydrolyze starch into reducing sugars such as glucose and

maltose. Suraini (2002) reported sago starch producing sugar not only may serve as a carbon source in the fermentation industries but also in the production of high-fructose syrup.

There is pretty important and essential to establish a mathematical model in order to predict the outcome of any industrial process. By doing this, one industrial process is not only time saving but also help to reduce operational cost and manpower. By definition, a mathematical model is generally an explicit, precise and accurate relationship between the reaction and environment. Scientifically, progress is made possible by comparing the results obtained from mathematical manipulations with the model with the results of experiments on the real system (Doran 1995). Hence, to acquire an effective modeling and simulation system, the underlying principles of numerical integration must be understood. The simulation provides a very simple and direct means of solving sets of simultaneous and algebraic equations and is therefore very convenient for the solution of biochemical engineering model.

In the sago starch-enzyme hydrolysis study, the model should describe the measured values as closely as possible and the parameters chosen should furthermore be open to enzyme kinetics interpretations. Apart from that, the model should also respond to corrective changes in order to accommodate the data obtained in the process experimentations (Posten 1994). The numerical method called Runge-Kutta algorithm (Press et al. 1986) is the most commonly software used to solve initial value problems particularly for ordinary differential equation (ODE). This method is momentous to family of explicit and implicit methods for an estimation of solution of ordinary differential equation. Since the Runge-Kutta Fehlberg 45 is a well-known algorithm to solve the initial value problems, it should be appropriated to use in this study to solve the initial value such as initial substrate or product concentration.

Since the degradation of sago starch into reducing sugars has great economic potential, intensive studies are needed to understand the many aspects of this complex reaction including its kinetics, effects of substrate concentration and enzyme loading. To the best of our knowledge, so far there is limited data on modeling of sago starch hydrolysis. Therefore, the finding outcome of this investigation will contribute or fill the research gap of sago starch study. Hence, this study was to develop the kinetics model for glucoamylase-catalyze hydrolysis from commercial sago starch by using proposed Michaelis-Menten-like equation and Runge-Kutta Fehlberg 45 algorithm.

## MATERIALS AND METHODS

### SUBSTRATE

The sago starch employed in the present study was from sago palm, *Metroxylon sago*. It was a gift from local commercial supplier, Wah Chang International (Malaysia). The starch powder was dried to constant weight at 60°C in oven prior to use. The sago starch consists of (in dry weight basis, %) amylose, 27; amylopectin, 73; lipids, 0.1; protein, 0.1; ash, 0.2 and phosphorus 0.2 (Swinkels 1985).

### ENZYME

The commercial glucoamylase enzyme (E.C 3.2.1.3) from *Aspergillus niger* was used as an sole enzyme in this research and was purchased from Sigma Aldrich (M) Sdn. Bhd. The specific activity of this enzyme was 31.2  $Umg^{-1}$  (Sigma-Alrich 1995).

### CHEMICAL REAGENTS

The chemical reagents employed for this investigation were of analytical grade with highest purity. They were included acetic acid ( $CH_3COOH$ ), 3,5-dinitrosalicylic acid ( $C_7H_4N_2O_7$ ), D-(+)-glucose ( $C_6H_{12}O_6$ ), liquefied phenol ( $C_6H_5OH$ ), potassium sodium tartrate tetrahydrate

( $C_4H_4KNaO_6 \cdot 4H_2O$ ), sodium acetate ( $CH_3COONa$ ), sodium hydroxide (NaOH), sodium sulfite ( $Na_2SO_3$ ).

### REACTION CONDITIONS

The substrate and enzyme solutions were prepared according to their optimal conditions which have been determined as described by Wee et al. (2011b). The reducing sugars were assayed according to Miller (1959) method. All experiments were performed in triplicates.

### CALCULATION OF $v$

The initial velocity,  $v$  of enzyme-substrate catalyzed reaction could be measured. It is usually expressed in differential equation as shown in (1)

$$v = \frac{dP}{dt} = -\frac{dS}{dt}, \quad (1)$$

where the  $P$  is the product formation (reducing sugars formed) and  $S$  is the substrate (sago starch) concentration, respectively. Both  $P$  and  $S$  possess a same unit in term of  $gL^{-1}$ ;  $t$  is the time in minute. The negative sign in (1) indicates that the substrate disappearance or product formation over the hydrolysis time. The (1) can also use to calculate  $v$  value of sago starch hydrolysis in present study.

### MATHEMATICAL MODELING

The modeling of sago starch hydrolysis in this study was performed using Polymath® 6.0 software. The analogue Michaelis-Menten equation

$$r = \frac{v_{max}S}{k_m + S}, \quad (2)$$

where  $S$  is substrate concentration ( $gL^{-1}$ );  $k_m$  is Michaelis constant, ( $gL^{-1}$ );  $v_{max}$  is maximum reaction rate ( $gL^{-1}min^{-1}$ ) and  $r$  is initial velocity ( $gL^{-1}min^{-1}$ ) was used and the model prediction was compared with the experimental results from sago starch hydrolysis reaction.

Meanwhile the renowned Michaelis-Menten equation is as followed:

$$v = \frac{v_{max}S}{k_m + S}. \quad (3)$$

In general, Michaelis-Menten equation has proven to be simple yet powerful approaches to describe the kinetic of most enzyme reactions (Lai et al. 2014). Hence, the modeling of sago starch hydrolysis with imitate in (2) should be satisfactory to fix the real Michaelis-Menten well.

The initial conditions for modeling such as time of reaction start,  $T_{initial}$ ; time of reaction end,  $T_{final}$ ; initial substrate concentration,  $S_{initial}$  and initial product concentration,  $P_{initial}$  were tabulated in Table 1 and optimal setting values were also shown in Table 2. The model with specified initial boundary conditions was solved by

TABLE 1. Initial conditions for the modeling

Initial conditions	Value	Unit
Time of reaction start, $T_{initial}$	0.000	minute
Time of reaction end, $T_{final}$	45.000	minute
Initial substrate concentration, $S_{initial}$	0.391	$gL^{-1}$
Initial product concentration, $P_{initial}$	$1.000 \times 10^{-6}$	$gL^{-1}$

TABLE 2. Differential and explicit equations for the modeling

Equation form	Description	Optimal setting values	Unit
Differential			
$\frac{dS}{dt} = -v$	volumetric rate of sago starch hydrolysis	-	$gL^{-1}min^{-1}$
Explicit			
$v_{max}$	maximum volumetric catalysis rate	0.424	$gL^{-1}min^{-1}$
$k_m$	Michaelis constant	2.324	$gL^{-1}$
$v = \frac{v_{max}S}{k_m + S}$	Michaelis-Menten equation	-	$gL^{-1}min^{-1}$

applying the Runge-Kutta Fehlberg 45 (RKF 45) algorithm. The RKF 45 algorithm was used to solve the ordinary differential equation (ODE) as shown in Table 2 with one differential and three explicit equations. The model was performed at enzyme amount and substrate concentration of  $0.2 Uml^{-1}$  and  $1.0 gL^{-1}$ , respectively. The data obtained were compared to the experimental results by using Polymath® 6.0 software.

## RESULTS AND DISCUSSION

The imitate Michaelis-Menten equation (2) was used as a model to predict the rate of sago starch hydrolysis by glucomaylase enzyme. The computational and experimental rates were compared and analyzed. Table 3 shows the experimental and computational rate at different along with regression coefficient,  $R^2$  values. The model describes the experimental data are very well with high

value of regression  $R^2 = 0.9643$ ,  $R_A^2 = 0.9583$ , and the variance,  $\delta = 0.0005$ . This indicates that only 3.57-4.17% of total variations were not explained by this model.

The prediction model shown the data obtained were quite closed to experimental results. When both data were graphically plot (Figure 1), we can see the pattern of experimental results (diamond shape) is in closed agreement with the predicted Michaelis-Menten model (line). Doran (1995) has also reported that Michaelis-Menten model is a satisfactory description of the kinetics model of many industrial enzymes. Hence we concluded that sago starch hydrolysis by glucoamylase was followed Michaelis-Menten kinetics and has been successfully modeled in ours study.

The enzymatic hydrolysis of sago starch was also simulated using Polymath® 6.0 software. The determination of reducing sugars concentration in the reaction mixture,  $Y_{p/s} = 0.39 \pm 0.05 gg^{-1}$  was used to calculate the remaining

TABLE 3. Experimental calculated data points

$S, gL^{-1}$	$r_{exp}, gL^{-1}min^{-1}$	$r_{calc}, gL^{-1}min^{-1}$
0.0	0.000±0.000	0.000
1.0	0.099±0.004	0.128
2.0	0.189±0.005	0.196
3.0	0.260±0.001	0.239
4.0	0.298±0.008	0.268
5.0	0.303±0.005	0.289
6.0	0.293±0.004	0.306
7.0	0.293±0.028	0.318

(maximum iteration of 64 runs, size of sample = 8, model variance = 2, independent variance = 1, iteration = 2);  $R^2 = 0.9642956$ ,  $R_A^2 = 0.9583449$ ,  $\delta = 0.0005305$ ,

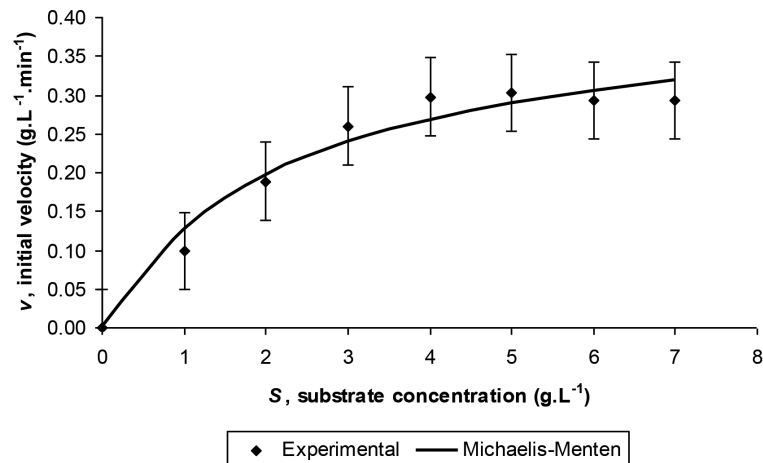


FIGURE 1. Comparison between experimental results and Michaelis-Menten model as a function of substrate concentration

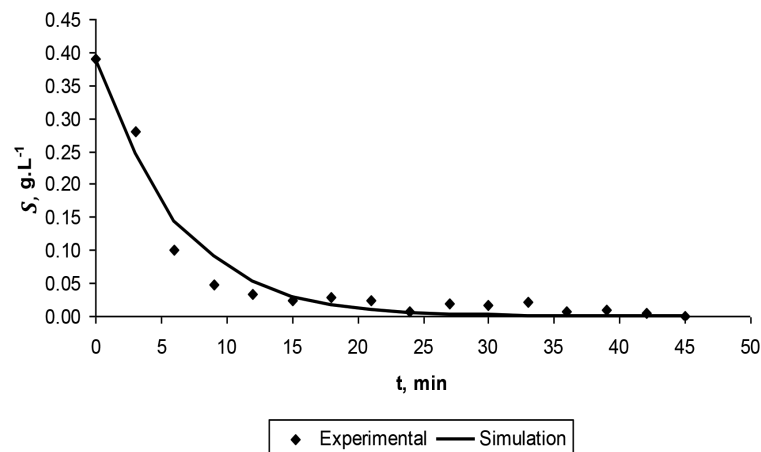


FIGURE 2. Time profile of experimental results and simulation model for remaining sago starch concentration,  $gL^{-1}$

sago starch concentration. The Runge-Kutta Fehlberg 45 (RKF 45) algorithm was used to solve ordinary differential equation (ODE) that consist of one differential and three explicit equations as displayed as Table 2.

Figure 2 illustrates the time profile of experimental (diamond shape) and proposed simulation model (line) for remaining sago starch concentration. The simulation predicted satisfactorily the remaining sago starch concentration. Consequently, it can be concluded that simulation results can be used to predict the remaining sago starch concentration in this research.

#### CONCLUSION

The kinetics of sago starch hydrolysis by glucoamylase obeyed Michaelis-Menten model. The computer simulation was successfully predicted the remaining of sago starch concentration in this study.

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